

Applicants: Nicole Suciu-Foca, et al.
Serial No.: 10/018,677
Filed: May 15, 2002
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Amendments to the Specification

Please replace the title appearing on page 1, lines 3 and 4 with the following amended title:

~~GENERATION OF ANTIGEN SPECIFIC T SUPPRESSOR CELLS FOR TREATMENT OF REJECTION~~ METHOD OF INDUCING ANERGIC T HELPER CELLS

Please replace the paragraph beginning on page 1, line 6 with the following amended paragraph:

This application claims priority and is a continuation-in-part application of U.S. Serial No. 09/333,809, filed June 15, 1999, now U.S. Patent No. 6,667,175, issued December 23, 2003, the contents of which is hereby incorporated by reference.

Please replace the paragraph beginning on page 4, line 8 with the following amended paragraph:

Understanding the mechanism which underlies the induction of immunologic tolerance is crucial to the development of strategies for treatment of auto-immune diseases and allograft rejection. Although the concept that T suppressor cells (Ts) downregulate the immune response has long been accepted, the existence of a distinct population of lymphocytes that mediates suppression has not been convincingly demonstrated. In previous studies, human T cell lines (TCLs) were utilized to analyze the suppressive effects of CD8⁺ CD28⁻ T cells in allogeneic, peptide specific and xeno-specific responses. In each case, CD8⁺ CD28⁻ T cells inhibit proliferation of CD4⁺ T helper lymphocytes (Th)

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with cognate antigen specificity. These CD8⁺ CD28⁻ T cells display the critical functional characteristics of T suppressor cells. Similar to the induction of CD8⁺ cytotoxic T cells (Tc) by Th, this process depends on antigen presenting cells (APC) acting as a "bridge" between MHC-class I specific CD8⁺ and class II specific CD4⁺ T cells. A possible explanation of Ts-mediated suppression is their ability to modulate the function of APCs. The fourth series of studies herein show that CD8⁺CD28⁻ Ts directly inhibit the CD40 signaling pathway of APC by a contact-dependent mechanism that renders bridging APCs incapable of inducing CD4⁺ T cell activation. The effects of Ts on the functional state of APC supports the concept that the order in which Ts and ~~The~~ Th cells interact with cognate APCs determines the functional outcome of immune responses.

Please replace the paragraph beginning on page 11, line 10 with the following amended paragraph:

This invention provides a method of inducing anergic T helper cells which comprises: a) incubating antigen presenting cells (APC) with allospecific T suppressor cells (Ts); b) overexpressing in the APC mRNA which encodes at least one monocyte inhibitory receptor (MIR), in a mixture of cells comprising the APCs from step (a), wherein overexpression of MIR transmits negative inhibitory signals to recruit an inhibitory signaling molecule, ~~thyrosine~~ tyrosine phosphatase SHP-1 such that the APC are rendered tolerogenic; and c) incubating the APCs from step (b) with T helper cells (Th) to induce Th anergy.

Please replace the paragraph beginning on page 20, line 4 with the following amended paragraph:

Figures 27A-27H. DRB Protein Sequences. Amino acid sequences of DRB protein correspond to hypervariable regions of HLS-DR B1 antigens. These antigens may be used as allopeptides for priming T suppressor cells (SEQ. ID. NOS:2-198).

Please replace the paragraph beginning on page 20, line 17 with the following amended paragraph:

Figure 29. Amino ~~acids~~ acid sequences of SLA DRA alleles. These amino acid sequences may be used for generating xenospecific human suppressor T cells in the methods described infra (SEQ. ID. NOS:199-204).

Please replace the paragraph beginning on page 20, line 21 with the following amended paragraph:

Figure 30. Amino ~~acids~~ acid sequences of SLA DRB alleles. These amino acid sequences may be used for generating xenospecific human suppressor T cells in the methods described infra (SEQ. ID. NOS:205-213).

Please replace the paragraph beginning on page 20, line 25 with the following amended paragraph:

Figure 31. Amino ~~acids~~ acid sequences of SLA DQA alleles. These amino acid sequences may be used for generating xenospecific human suppressor T cells in the methods described infra (SEQ. ID. NOS:214-219).

Please replace the paragraph beginning on page 20, line 29 with the following amended paragraph:

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Figure 32. Amino ~~acids~~ acid sequences of SLA DQB alleles. These amino acid sequences may be used for generating xenospecific human suppressor T cells in the methods described infra (SEQ. ID. NOS:220-228).

Please replace the paragraph beginning on page 88, line 3 with the following amended paragraph:

A chimeric peptide tat-DR4, comprising residues 49-57 of HIV-tat and residues 64-88 of DRB1*0401 was purchased ~~from~~ from Chiron Technology, Australia. The purity of the peptide was >85% as determined by reverse-phase HPLC. The amino acid sequence of this peptide is as follows: RKKRRQRRRQKDLLEQKRAAVDTYCRHNYGVGES (SEQ. ID. NO:1).

Please replace the paragraph beginning on page 104, line 32 with the following amended paragraph:

The aim of the present study was to investigate whether the suppressor effect requires the concomitant interaction between Ts, ~~The~~ Th and APCs or sequential two cell interactions (first, between Ts and APCs and next, between "suppressed" APCs and ~~The~~ Th) and whether it is mediated by inhibition of the CD40-signaling pathway.

Please replace the paragraph beginning on page 118, line 3 with the following amended paragraph:

In the first through fourth series of experiments, we identified and characterized human antigen specific T suppressor cells (Ts). It was shown that Ts inhibits the costimulatory activity of APC blocking NF- κ B activation and transcription of costimulatory molecules. To explore the

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underlying mechanism we used for allostimulating peripheral blood B cells or cells from the dendritic cell line KG-1. Total RNA prepared from KG-1 or from B cells that have been exposed to allospecific Th, Ts or Th/Tz mixtures for 12 hours was used in a cDNA micro-array system to identify genes which are differentially expressed in APC. Although transcription of a wide array of genes was suppressed, expression of 10-15 genes was up-regulated >2-3 fold in APC cocultured for 12 hours with Ts or Ts/Th mixtures. Included in this latter group are the Monocyte Inhibitory Receptor (MIR-10 or ILT4), ILT2 (MIR7), and ILT3. MIR-10, MIR7 (ILT2) and ILT3 belong to a family of leukocyte inhibitory receptors (LIRs) which bear homology to killer inhibitory receptors (KIRs). These molecules interact with MHC-class I molecules via Ig-like domains and regulate negatively the activation of APC, recruiting an inhibitory signaling molecule, ~~thyrosine~~ tyrosine phosphatase SHP-1. These data indicate that Ts-induced suppression of APC is based on an active mechanism by up-regulating the expression of a class of inhibitory receptors which transmit negative inhibitory signals in APC. Ts provides an essential regulatory mechanism through which immune tolerance can be achieved.

Please insert the Sequence Listing, annexed hereto as Exhibit A into the specification after the paragraph beginning on page 118, line 38.